

STUDIES OF SEX DETERMINATION
AND SEX PRODUCTION IN
HYDATINA SENTA.

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Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy,
in the Faculty of Pure Science, Columbia University.

REPRINTED FROM

THE JOURNAL OF EXPERIMENTAL ZOÖLOGY

Volume V, No. 1, pp. 1-25.

Volume VI, No. 1, pp. 125-145.



BALTIMORE, MD., U. S. A.

November, 1907.

January, 1909.

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I INTRODUCTION

On account of the supposed influence of external factors in determining sex in *Hydatina senta*, this rotifer has attracted much interest in recent years. As is well known *Hydatina* produces three kinds of eggs, viz: (1) parthenogenetic eggs which develop into females; (2) smaller parthenogenetic eggs which develop into males; and (3) fertilized eggs which develop into females. Each female produces only one of these three kinds of eggs. Thus three types of females may be distinguished, viz: (1) females which produce females parthenogenetically, or female-laying females, ♀ ♀; (2) females which produce males parthenogenetically, or male-laying females, ♂ ♀; and (3) the sexual females that lay fertilized eggs.

Both female-laying females and male-laying females can be impregnated by males, but on the former, impregnation is supposed to have no effect. If the male-laying females are impregnated by the male in the first few hours after they leave the egg, such females produce fertilized eggs instead of parthenogenetic male eggs, thus showing that male-laying females can develop into sexual females that lay fertilized eggs.

The female-laying female can produce a family of daughter-females, some of which may lay female eggs and others may lay male eggs.

With the view of finding out the ratio in which these two classes of daughter-females are produced under various conditions I have carried out the experiments to be described.

Maupas found that a temperature of 26° to 28° C. would produce as high as 95 per cent of male-laying females while a temperature of about 14° C. would produce as low as 5 per cent of male-laying females.

Nussbaum, on the contrary, came to the conclusion that nutrition and not temperature is the sex controlling factor. He found that by starving the young females for the first few hours after they emerge from the egg they would produce a high percentage of males, but if they were fed at the time they leave the egg they produce a high percentage of females.

Punnett has carried out a few experiments along the lines laid down by Maupas and Nussbaum and finds that neither temperature nor nutrition is influential in determining the sex. He finds, on the contrary, that there are definite "sex strains." Some strains produce 40 to 50 per cent of males, others produce a very low percentage, 2 to 5 per cent, while others produce no males at all, although reared through as many as seventy-two generations.

The greater part of the work of the present paper was planned and begun in the spring of 1906, under the direction of Prof. T. H. Morgan, before the results of Punnett were published. Not knowing how to obtain proper food cultures the rotifers all died in July and the continuation of the experiments was deferred until October, 1906.

II MATERIAL AND METHODS

In the latter part of April, 1906, *Hydatina senta* was discovered in great numbers in a small pool on the Palisades of New Jersey near Grantwood. The pool was fed by a little stream or ditch which carried away the drainage from several cottages. The ditch was an extremely favorable place for the growth of *Euglena viridis* which collected in large patches on the sides and bottom. Immense numbers of *Euglena* floated down into the pool at the end of the ditch and served as food for the rotifers which abounded there in countless thousands. Sometimes as many as 150 to 250 individuals could be drawn up by a pipette in a few cc. of water.

About May 15 the pool dried up completely. The ditch still contained water but no rotifers were found in it after May 20. At this time there were innumerable larvæ of insects in the ditch and perhaps they exterminated the rotifers by feeding upon them.

In all experiments each individual female was isolated in a square or round watch glass which contained about 5 cc. of water and fed with *Euglena*, other protozoa and bacteria.

In order to obtain the *Euglena* and other protozoa a culture of horse manure and water (one to two ounces to a quart) was made, inoculated with *Euglena* and allowed to stand for two to three weeks at room temperature. At the end of this time the green coating of algæ, *Euglena*, etc., could be removed from the sides of the glass jar and served as an excellent food for the rotifers.

Great care was taken to keep these food cultures uncontaminated by rotifers. All watch glasses were placed in hot water after each experiment in order to destroy all eggs which adhered to the sides, thus preventing contamination of the following experiments by eggs of the preceding ones.

The experiments at temperature of 24° to 29° C. were conducted in an incubator. Those at a temperature of 20° to 22° C. were conducted on the laboratory tables at room temperature, while those at a temperature of 14° to 15° C. were carried on in an ice chest.

These rotifers are exceedingly hardy and can be very easily kept in the laboratory throughout the year. In May of 1906 a *Euglena* culture was prepared in a glass jar containing 2000 cc. of water

and a few rotifers put into it. The jar was covered so as to prevent evaporation of the water. Rotifers have lived in it to this time, April, 1907, although no more food material has ever been added. It is absolutely necessary that the surface of the water be free from a scum for the rotifers will die within a few hours if it is present. It is safer, in order to keep the surface free, to tie the horse manure in a muslin cloth and place it in a well covered jar nearly filled with water.

III INFLUENCE OF TEMPERATURE

I *Maupas' Experiments*

The experiments of Maupas were so briefly described that it is very difficult to understand clearly how he obtained his results.

Nussbaum and Punnett are inclined to believe he determined that a female was a male-laying or a female-laying individual by the size of the eggs that she produced. Small eggs being assumed always to give rise to males while larger eggs give rise to females. Nussbaum has measured a series of both male and female eggs and found that in some instances the two kinds of eggs over-lap in size. Thus he points out an error through which Maupas' results might have been obtained.

Isolating and counting the eggs of this rotifer would be exceedingly tedious and require almost constant attention. As the sexes can be readily distinguished at any period and as it requires only 36 to 48 hours for a female to mature and produce eggs it seems to me extremely probable that Maupas must have allowed some at least of the eggs to hatch before recording his results.

As his experiments are so few and briefly described it may be well to present them here in order that they may be compared with and interpreted by my own. Experiment I. Lot A, temperature 26° to 28° C. Five female-laying female sisters produced 104 eggs; 97 per cent developed into male-laying females. Lot B, temperature 14° C. Five other female-laying females, which were sisters of lot A, produced 260 eggs; 5 per cent developed into male-laying females.

Experiment II, temperature 14°C . Five female-laying females, kept from the time of hatching at this temperature, produced 110 eggs; 24 per cent developed into male-laying females.

The same five female-laying females were then placed at a temperature of 26° to 28°C . and produced 81 per cent male-laying females.

Experiment III, temperature 14°C . Six female-laying females which had been kept at this temperature from the time of hatching produced 34 eggs, of which 12 per cent developed into male-laying females.

The same six female-laying females were then placed at a temperature of 26° to 28°C . and allowed to produce 44 eggs, of which 95 per cent developed into male-laying females. These six females were alternately placed at 14°C . and 28°C . several times and always gave a high percentage of male-laying females at the higher temperature.

2 *Author's Experiments*

a Temperature 20° to 22°C .

Experiment I, October 24, 1906. A female-laying female was isolated from a jar which was stocked with rotifers collected October 2, from the same pool in which the animals were found in the preceding spring.

This strain was carried through twelve generations and the percentage of male-laying females determined. Each female was supplied with an abundance of food from the time of hatching, isolated in a separate watch-glass, and kept upon the laboratory table at room-temperature.

Table I gives the ratio of the mother individuals producing male and female offspring in the 3264 daughter-females of 95 female-laying females in the twelve generations.

This experiment was made in order to obtain the percentage of male-laying females produced at room temperature of 20° to 22°C ., in order to be able to have some standard percentage of male-laying females with which to compare the results of the experiments conducted at lower and higher temperatures.

TABLE I

Record of the production of male-laying and female-laying females among the 3264 daughter-females of 95 female-laying mothers.

Temperature 20° to 22° C.

Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀	Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
			♂ ♀	♀ ♀					♂ ♀	♀ ♀	
I	1	31	15	16	48+	X	3	32	8	24	25
							4	15	3	12	20
II	1	28	0	28	0		5	43	8	35	18+
	2	53	8	45	15+		6	48	11	37	22+
	3	27	4	23	14+		7	25	0	25	0
III							8	38	15	23	39+
	1	44	17	27	38+		9	17	4	13	23+
	2	46	14	32	30+		10	48	15	33	31+
IV							11	48	16	32	33+
	1	51	1	50	1+		12	43	4	39	9+
	2	47	15	32	31+		13	44	10	34	22+
	3	52	16	36	30+		14	49	14	35	28+
V	4	35	7	28	20		15	45	18	27	40
							16	16	6	10	37+
	1	47	0	47	0		17	45	4	41	8+
	2	44	2	42	4+		18	50	7	43	14
VI	3	41	1	40	2+		19	32	4	28	12+
	4	45	0	45	0		20	21	0	21	0
							21	21	4	17	19+
	1	32	12	20	37+		22	20	2	18	10
	2	45	4	41	8+		23	25	0	25	0
VII	3	36	0	36	0	XI	1	49	8	41	16+
	4	47	7	40	14+						
	5	48	22	26	45+	XII	1	38	3	35	7+
							2	45	9	36	20
	1	30	15	15	50		3	48	4	44	8+
VIII	2	35	1	34	2+		4	31	5	26	16+
	3	27	0	27	0		5	39	13	26	33+
	4	24	0	24	0		6	31	10	21	32+
	5	19	5	14	26+		7	54	10	44	18+
							8	45	6	39	13+
IX	1	41	16	25	39+		9	35	11	24	31+
	2	17	1	16	5+		10	43	5	38	11+
	3	47	18	29	38+		11	38	3	35	7+
	4	26	0	26	0		12	27	9	18	33+
	5	38	0	38	0		13	42	16	26	38+
X	1	24	1	23	4+		14	34	1	33	2+
							15	43	15	28	34+
	1	49	13	36	26+		16	37	6	31	16
	2	45	10	35	22+		17	31	5	26	16+

TABLE I—Continued

Temperature 20° to 22° C.

Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀	Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
			♂ ♀	♀ ♀					♂ ♀	♀ ♀	
XII	18	26	7	19	22+	XII	30	16	5	11	31+
	19	33	3	30	9+		31	9	1	8	11+
	20	44	3	41	6+		32	18	8	10	44+
	21	20	0	20	0		33	20	6	14	30
	22	45	9	36	20		34	47	14	33	29+
	23	31	11	20	35		35	19	4	15	21+
	24	28	10	18	35+		36	26	8	18	30+
	25	15	3	12	20		37	11	0	11	0
	26	34	1	33	2+		38	37	11	26	29+
	27	15	0	15	0		39	34	19	15	55+
XII	28	14	3	11	21+		40	25	6	19	24
	29	6	1	5	16+		41	35	4	31	11+

The nature of the sex-producing power of the daughter-females of each individual mother is given separately in order to show that the ratio between the daughter-females producing male and female offspring varies with different mother-individuals, and also varies as much in the daughter females of sister-mother-individuals.

TABLE II

Summary of each generation in Table I and also the final summary of all the generations taken together.

Temperature 20° to 21° C.

Gen.	♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀	Gen.	♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
			♂ ♀	♀ ♀					♂ ♀	♀ ♀	
I	1	31	15	16	48+	VII	5	135	21	114	15+
II	3	108	12	96	11+	VIII	5	169	35	134	20+
III	2	90	31	59	34+	IX	1	24	1	23	4+
IV	4	185	39	146	21+	X	23	819	176	643	21+
V	4	177	3	174	1+	XI	1	49	8	41	16+
VI	5	208	45	163	21+	XII	41	1269	268	1001	21+
							95	3264	654	2610	20+

Table II gives the summary of each generation and the final summary of the twelve generations.

In this experiment 95 mother-individuals produced 3264 daughter-females of which 20 + per cent were male-laying females.

It will also be noted that the percentage of male-laying females varied in the different generations from 1 + per cent to 48 + per cent regardless of the number of isolations in each generation.

b Temperature 25° to 29° C.

Experiment II, October 26. Two female-laying females were

TABLE III

Record of the production of male-laying and female-laying females among the 208 daughter-females of 26 female-laying mothers.

Temperature 25° to 26° C.

Temperature 26° to 29° C.

	Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀		Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
				♂ ♀	♀ ♀						♂ ♀	♀ ♀	
Strain IV	I	1	17	0	17	0	Strain III	IX	1	4	0	4	0
	II	1	6	1	5	16+			2	4	0	4	0
Strain III	I	1	17	0	17	0	X		1	3	0	3	0
	II	1	13	4	9	30+			2	5	0	5	0
									3	1	1	0	100
									4	1	0	1	0
									5	8	2	6	24+
									6	6	1	5	16+
									7	8	2	6	24+
									8	3	0	3	0
								9	5	2	3	40	
								10	4	2	2	50	
								11	4	0	4	0	
								12	9	0	9	0	
								13	1	1	0	100	
								14	10	3	7	30	
								15	1	1	0	100	
									26	208	46	162	22+

isolated from the same stock jar in a similar manner as in Experiment I, and placed in an incubator. Two generations from one

individual, strain IV, and six generations from the other individual, strain III, were recorded. Six generations were kept at a temperature of 25° to 26° C. and two generations at 26° to 29° C.

Table III gives the results of the experiment obtained at this higher temperature. The 26 female-laying mothers from eight generations, and from two strains, produced 208 daughter-females of which 22 + per cent were male-laying females. This percentage is practically the same as that obtained at room temperature.

c Temperature 14° to 15° C.

Experiment III, October 8. A female-laying female was isolated from stock jar as in Experiment I, and placed in an ice chest. A record of only a few of her offspring was kept and is shown in Table IV.

TABLE IV

Record of the production of male-laying and female-laying females among the 167 daughter-females of 7 female-laying mothers.

Temperature 14° to 15° C.

No. of ♀ ♀ mother	Eggs laid	Offspring			Per cent ♂ ♀
		Fed	♂ ♀	♀ ♀	
1	48	47	11	36	23+
2	50	28	4	24	14+
3	30	20	3	17	15
4	22	9	2	7	22+
5	41	19	5	14	26+
6	36	15	0	15	0
7	51	29	10	19	34+
7	278	167	35	132	20+

Out of 167 daughter-females from 7 different mothers 20 + per cent were male-laying females. The mother-individuals were reared at this low temperature as well as the daughter-females.

The percentage of male-laying females is about the same as that obtained at temperatures 20° to 22° C. and 25° to 29° C.

The foregoing results agree with those obtained by Nussbaum and Punnett but seem contrary to Maupas' results.

IV THE RELATIVE NUMBER OF EGGS WHICH A MALE-LAYING FEMALE AND A FEMALE-LAYING FEMALE PRODUCE

It seems evident from Maupas' account of his own experiments that he did not isolate each female-laying mother and each one of her daughter-females but kept the female-laying mothers together in one dish and their daughter-females together in another dish.

If it is assumed that Maupas made no mistake in determining the sex character of the eggs before they hatched, or even that he allowed all eggs to hatch before he recorded their sex character, his results can be easily explained.

TABLE V

The number of eggs laid by each of 13 sisters, of which 6 were male-laying and 7 were female-laying, showing that the average number of eggs laid by each of the two kinds of females is very nearly the same.

Temperature 20° to 22° C.

13 sisters

♂ ♀ Mother	♀ Eggs	Av.		♀ ♀ Mother	♀ Eggs	Av.
1	46			1	47	
1	50			1	38	
1	47			1	37	
1	46			1	43	
1	41			1	38	
1	31			1	45	
				1	38	
6	261	43½		7	268	40⅘

He gives no results of experiments conducted at a temperature midway between 14° and 28° C. but only results obtained at these two extremes. The results that were obtained at 14° C. may very likely be identical with those that could have been obtained at a room temperature around 20° C.

Maupas recognized the fact that male-laying females produce eggs faster than female-laying females but makes no mention of the number of eggs that each kind of female may produce at different temperatures. He seems to assume that they always pro-

duce about an equal number, 40 to 50 each, but the following experiments will show the error of this assumption.

1 Temperature 20° to 22° C.

Experiment IV, November 5. Of 13 sister individuals kept at room temperature and with the same amount of food 6 produced male eggs and 7 produced female eggs. The average number of eggs produced by each female was nearly the same. The results are shown in Table V.

2 Temperature 24° to 29° C.

Experiment V, November 9. Three lots of sister-individuals from three different mother-individuals were kept in an incubator

TABLE VI

Record of the number of eggs laid by 11 sisters, of which 6 were male-laying and 5 were female-laying, showing that the average number of eggs produced by the male-laying females is about two times as great as the average number produced by the female-laying females.

Temperature 24° to 25° C.

11 sisters

♂ ♀ Mother	♂ Eggs	Av.		♀ ♀ Mother	♀ Eggs	Av.
1	37			1	16	
1	35			1	17	
1	26			1	16	
1	38			1	11	
1	26			1	10	
1	22					
6	184	30 $\frac{2}{3}$		5	70	14

and the number and sex character of the eggs that each produced was very carefully noted. The results are shown in Tables VI, VII and VIII. The records of the individuals in Tables VI and VII were taken at the same temperature of 24° to 25° C. while those of Table VIII were taken at a higher temperature of 26° to 29° C.

These tables show a decided change in the ratio between the number of eggs produced by a male-laying female and a female-laying female. As the temperature is raised the female-laying

TABLE VII

Record of the number of eggs laid by 21 sisters, of which 9 were male-laying and 12 were female-laying, showing the average number of eggs produced by the male-laying females is about two times as great as the average number produced by the female-laying females.

Temperature 24° to 25° C.

21 sisters

♂ ♀ Mother	♂ Eggs	Av.		♀ ♀ Mother	♀ Eggs	Av.
1	25			1	6	
1	39			1	14	
1	34			1	16	
1	37			1	11	
1	28			1	10	
1	25			1	12	
1	29			1	12	
1	23			1	9	
1	22			1	17	
				1	14	
				1	16	
				1	21	
9	261	29 $\frac{1}{3}$		12	158	13 $\frac{1}{6}$

TABLE VIII

Record of the number of eggs laid by 14 sisters, of which 2 were male-laying and 12 were female-laying, showing that the average number of eggs produced by the male-laying females is nearly four times as great as the average number produced by the female-laying females.

Temperature 26° to 29° C.

14 sisters

♂ ♀ Mother	♂ Eggs	Av.		♀ ♀ Mother	♀ Eggs	Av.
1	23			1	3	
1	19			1	5	
				1	1	
				1	8	
				1	6	
				1	8	
				1	3	
				1	5	
				1	4	
				1	4	
				1	9	
				1	10	
2	42	21		12	66	5 $\frac{1}{2}$

females produce fewer and fewer eggs while the decrease in the number of eggs produced by male-laying females is not as great.

Table IX shows a rough approximation of the ratio in which the male and female eggs are produced at these different temperatures.

TABLE IX

The approximate ratios in which the males and females are produced at different temperatures, as seen in Tables V-VIII.

♂	♀
1	1 Temp. 20° to 22° C. Table V
2	1 Temp. 24° to 25° C. Table VI-VII
4	1 Temp. 26° to 29° C. Table VIII

From the foregoing experiments and tables it is evident that temperature has nothing to do directly with determining sex in *Hydatina senta* but indirectly it determines the number of each sex produced by regulating the number of eggs that each kind of female lays. At a temperature of 20° to 22° C. the male-laying and female-laying females lay about the same number of eggs each, but at a higher temperature of 26° to 29° C. the male-laying females lay about four times as many as the female-laying females.

V EARLY PRODUCTION OF MALE-LAYING FEMALES IN A FAMILY OF DAUGHTER-FEMALES

None of the previous workers with *Hydatina senta* have isolated the eggs of a female-laying female in the order in which they were produced to determine whether there is any tendency for the earlier laid eggs to produce more male-laying females than the later laid eggs.

In my experiments in which all the daughter-females of each individual mother were carefully isolated and the sex character of their immediate offspring was recorded it is clearly shown that the male-laying daughter females appear among the earlier ones in the family rather than among the later ones.

In Diagram 1 the plotted line indicates the production of male-laying females among the 472 daughter-females of eleven mother-

individuals, each one of which produced 40 to 44 (average $42\frac{10}{11}$) eggs. The eggs were allowed to hatch in the dish with each mother and the young daughter-females isolated soon after hatching. Their different sizes would indicate their relative ages and thus the approximate order in which the eggs were produced. The young daughter-females were isolated in lots from 1 to 8. This manner of isolation is subject to some error but on the whole gives a fairly good approximation of the truth.

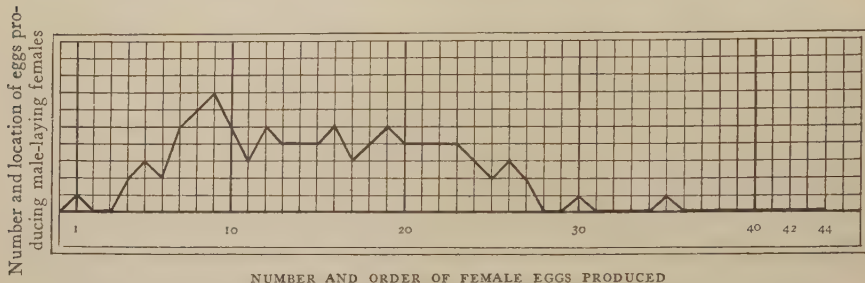


Diagram 1 Record of the egg production of 11 female-laying females from Table I, showing in which part of the egg laying period the male-laying females were produced. Each female laid 40 to 44 (average $42+$) eggs. Of the 472 daughter-females 20+ per cent were male-laying females.

Nearly all of the male-laying females were produced among the first 28 eggs laid. Only two male-laying females were produced from the twenty-ninth to the forty-second laid eggs. Of the daughter-females 20+ per cent were male-laying females.

Diagram 2. This is to show the same point as Diagram 1.

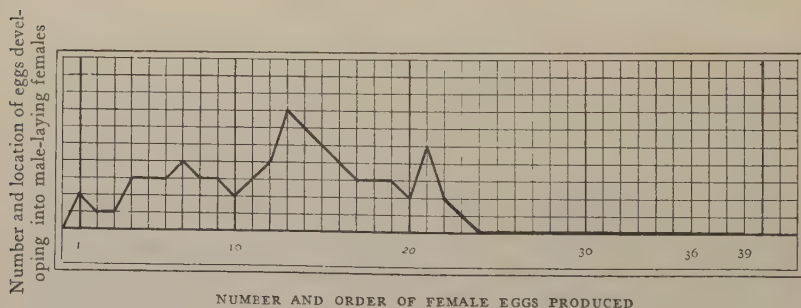


Diagram 2 Record of the egg production of 12 female-laying females from Table I, showing in which part of the egg laying period the male-laying females were produced. Each female laid 35 to 39 (average $36+$) eggs. Of the 441 daughter-females 16+ per cent were male-laying.

Twelve other female-laying females, each of which laid 35 to 39 (average 36+) eggs, produced all their male-laying daughter-females among the first twenty-four eggs laid. This is a clearer case than Diagram 1, because there are no scattering male-laying females among the later produced eggs. Of the daughter-females 16+ per cent were male-laying females.

In these two diagrams the mother-individuals were not specially selected but the record of all mothers, in Table I, producing 40 to 44 daughter-females, is shown in one diagram and the record of all mothers, in Table I, producing 35 to 39 daughter-females is shown in the other diagram. The numbers 35 to 39 and 40 to 44 were chosen because they seemed more likely to be the normal than a higher one.

These results, together with those obtained at different temperatures throw a great deal of light upon Maupas' results. In his experiments the highest percentages of males was always obtained from mothers which developed from early laid eggs.

In Table I it is seen that an individual mother may produce 0 to 40+ per cent of daughter male-laying females. This fact must also be taken into account when explaining Maupas' few experiments.

VI INFLUENCE OF FOOD

Nussbaum supported Maupas' conclusions that external factors can change the sex ratio in *Hydatina* but explains this change as being due to poor nutrition of the females and not due directly to the influence of temperature. At the higher temperature the processes of metabolism are taking place so rapidly that the animals cannot eat food and assimilate it fast enough to prevent their tissues from being in a semi-starved condition. Nussbaum's experiments seemed to show evidence that young females which are starved for several hours as soon as they leave the egg produce a higher percentage of males than those that are fed from the moment they hatch.

In many of his experiments he kept many individual females together and did not follow the history of each individual separately.

Punnett has pointed out that all the starved females of Nussbaum's experiments did not produce males, which invalidates his general conclusions.

Punnett has isolated female eggs of a "pure female strain," and after they hatched starved the young females from 2 to 20 hours but no males ever appeared, although the young females were starved for several consecutive generations.

I have followed the history of many females which have been starved for several hours immediately after hatching at a temperature ranging from 14° to 29° C. and have found no trace of evidence that a higher percentage of male-laying females is produced.

I Temperature 20° to 22° C.

Experiment I. Sixty-two eggs were selected at random from the sets of eggs produced by four female-laying females. They

TABLE X

Record of the sex character of the eggs laid by 27 sisters, 15 of which were without food for the first 6 to 26 hours after hatching and 12 were abundantly supplied with food from the moment they hatched.

Temperature 20 to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
2	6		♀
2	10		♀
5	11		♀
1	12		♀
1	16		♀
2	21		♀
1	23	♂	
1	26	♂	
2	fed	♂	
10	fed		♀

TABLE XI

Record of the sex character of the eggs laid by 45 sisters, 11 of which were without food for the first 21 to 26 hours after hatching and 34 were abundantly supplied with food from the moment they hatched.

Temperature 20 to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
11	21-26		♀
13	fed	♂	
21	fed		♀

were placed in "Great Bear" spring water, such as is sold in New York City for drinking purposes, and allowed to hatch. After

hatching each daughter-female was kept in this water without food from 6 to 71 hours.

Of 109 daughter-females from the same mother as the above, 62 were well supplied with food from the moment they hatched.

Tables X to XIII give the detailed results and Table XIV gives the summary. The mother-individuals of Tables X and XII

TABLE XII

Record of the sex character of the eggs laid by 53 sisters, 31 of which were without food for the first 11 to 59 hours after hatching and 22 were abundantly supplied with food from the moment they hatched.

Temperature 20 to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
1	11		♀
1	13		♀
2	14		♀
1	15		♀
1	21		♀
5	36	♂	
7	36		♀
1	38	♂	
2	38		♀
1	42		♀
1	47	♂	
4	47		♀
2	50		♀
1	54		♀
1	59		♀
1	fed	♂	
21	fed		♀

TABLE XIII

Record of the sex character of the eggs laid by 46 sisters, 5 of which were without food for the first 50 to 71 hours after hatching and 41 were abundantly supplied with food from the moment they hatched.

Temperature 20° to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
2	50	♂	
1	50		♀
1	71		♀
1	71	♂	
11	fed	♂	
30	fed		♀

were sisters. The 11 sisters-individuals of Table XI were starved in filtered boiled spring water placed in sterilized test tubes with cotton stoppers.

The percentage of male-laying females among all the starved daughter-females is slightly lower than that of those which were fed.

TABLE XIV

Summary of Tables X to XIII, showing the percentage of male-laying females that occurred among the females which were without food for the first 6 to 71 hours after hatching, and also the percentage of male-laying females which occurred among the females that were abundantly supplied with food from the moment they hatched.

Temperature 20° to 22° C.

Individuals	Starved from time of hatching	Character of eggs produced		Per cent ♂ ♀
		♂	♀	
	<i>hours</i>			
62	6-71	12	50	19 +
109	fed	27	82	24 +
171		39	132	22 +

2 *Temperature 14° to 15° C.*

Experiment II, October 29. Two female-laying females were reared at this temperature and their daughter-females isolated. Thirty-five daughter-females were without food from 11 to 64 hours after they left the egg, and 49 were abundantly supplied with food as soon as they hatched.

The detailed results are shown in Tables XV and XVI while Table XVII gives the summary.

The difference between the percentage of males produced by those starved and those fed is not very great and probably means nothing.

3 *Temperature 25° to 26° C.*

Experiments III, October 31. Twenty-six female eggs from several individuals were produced at this temperature and as soon as they hatched the young females were starved from 1 to 13 hours. 19+ per cent of these starved females produced male eggs.

Tables XVIII and XIX give the detailed history and Table XX gives the summary.

These three experiments, including Tables X to XX, clearly demonstrate that food has no influence in determining whether a female shall produce male or female offspring.

TABLE XV

Record of the sex character of the eggs laid by 49 sisters, 19 of which were without food for the first 11 to 41 hours after hatching and 30 were abundantly supplied with food from the moment they hatched.

Temperature 14° to 15° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	hours		
4	11		♀
1	20		♀
2	20	♂	
3	21		♀
1	23		♀
5	25		♀
1	25	♂	
1	27		♀
1	41		♀
4	fed	♂	
26	fed		♀

TABLE XVI

Record of the sex character of the eggs laid by 35 sisters, 16 of which were without food for the first 20 to 64 hours after hatching and 19 were abundantly supplied with food from the moment they hatched.

Temperature 14° to 15° C.

Sister-individuals	Starved from time of hatching	Character of egg produced	
		♂	♀
	hours		
2	20		♀
1	21	♂	
2	21		♀
2	23		♀
1	46	♂	
1	46		♀
1	48		♀
1	53	♂	
1	53		♀
1	58		♀
1	61	♂	
1	61		♀
1	64	♂	
5	fed	♂	
14	fed		♀

VII MALE AND FEMALE STRAINS

Punnett says: "My experiments have led me to the conclusion that among the rotifers I used were certainly three different types of thelytokous (female-laying) females, viz:

A Females producing a high percentage of arrenotokous (male-laying) females.

B Females producing a low percentage of arrenotokous females.

C Purely thelytokous females producing no arrenotokous females" (p. 226).

TABLE XVII

Summary of Tables XV to XVI, showing the percentage of male-laying females that occurred among the females which were without food for the first 11 to 64 hours after hatching, and also the percentage of male-laying females which occurred among the females that were abundantly supplied with food from the moment they hatched.

Temperature 14° to 15° C.

Individuals	Starved from time of hatching	Character of eggs produced		Per cent ♂ ♀
		♂	♀	
35	11-64	8	27	22 +
49	fed	9	40	18 +
84		17	67	20 +

TABLE XIX

Record of the sex character of the eggs laid by 19 individuals females which were without food for the first 1 to 13 hours after hatching.

Temperature 25° to 26° C.

Individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
1	1		♀
2	2		♀
1	3	♂	
3	3		♀
1	4	♂	
1	5	♂	
2	5		♀
2	6		♀
1	8	♂	
3	10		♀
2	13		♀

TABLE XVIII

Record of the sex character of the eggs laid by 15 sisters, 7 of which were without food for the first 7 to 13 hours after hatching and 8 were abundantly supplied with food from the moment they hatched.

Temperature 25° to 26° C.

Sister individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
2	7		♀
2	10		♀
1	10	♂	
2	13		♀
1	fed	♂	
7	fed		♀

TABLE XX

Summary of Tables XVIII to XIX, showing the percentage of male-laying females that occurred among the 26 females that were without food for the first 1 to 13 hours after hatching.

Temperature 25° to 26° C.

Individuals	Starved from time of hatching	Character of eggs produced		Per cent ♂ ♀
		♂	♀	
	<i>hours</i>			
26	1-13	5	21	19 +

Punnett realized that these conclusions were based on rather scanty data. His data can be shown to be entirely insufficient. His type *A* is based upon only one experiment which extended through 23 generations and included 109 individuals. 42 + per cent of these 109 individuals were male-laying females.

Type *C* is based upon much more evidence, but it is not sufficient to warrant a decisive conclusion.

In October, 1906, I started a strain or pedigree culture which extended through 62 generations including 167 mother female-laying females and 3959 daughter-females. This strain was kept at room temperature of 20° to 22° C. Its history is recorded in Tables I and XXI.

Table I. Out of 3264 daughter-females from 95 mother-individuals which extended through 12 generations 20 + per cent were male-laying females.

Table XXI. In 15 generations, XIII to XXVIII, including 76 daughter-females from 15 mother-individuals only 9 + per cent were male-laying females.

In 17 generations, XXIX to XLV, including 208 daughter-females from 17 mother-individuals no male-laying females appeared. In generation XLVI the first 327 daughter-females from 18 mother-individuals yielded 48 + per cent of male-laying females.

The next 11 generations XLVII to LVII including 58 daughter-females from 11 mother-individuals gave 29 + per cent male-laying females.

These results show that a strain producing a higher percentage of male-laying females can develop into a strain yielding a much lower percentage, or even into a strain yielding no male-laying females at all. Furthermore, the apparently pure female-laying female strain can develop into one which will give a very high percentage of male-laying females.

Thus the three strains or types of Punnett can be found in one strain and each is capable of giving rise to the other types according as the data is scanty or extensive.

The high percentage of male-laying females in generation XLVI can be readily explained by the results shown in Diagrams 1 and 2 which clearly demonstrate that the male-laying females are pro-

duced earlier in a set of eggs than are the majority of the female-laying females. In this generation the 18 mothers produced only an average of 18 + eggs each, because the experiment was discontinued at this point.

TABLE XXI

Continuation of the strain of which the beginning is recorded in the 12 generations of Table I.

Gen.	♀ ♀ Mother	Daugh- ter ♀ isolated	♂ ♀	♀ ♀	Gen.	♀ ♀ Mother	Daugh- ter ♀ isolated	♂ ♀	♀ ♀
XIII	1	6	1	5	XXXVIII	1	37	0	37
XIV	1	6	1	5	XXXIX	1	6	0	6
XV	1	6	0	6	XL	1	26	0	26
XVI	1	6	0	6	XLI	1	25	0	25
XVII	1	2	0	2	XLII	1	14	0	14
XVIII	1	1	0	1	XLIII	1	18	0	18
XIX	1	5	0	5	XLIV	1	17	0	17
XX	1	6	0	6	XLV	1	18	0	18
XXI	1	6	0	6	XLVI	18	327	160	167
XXII	1	6	3	3	XLVII	1	1	0	1
XXIII	1	5	0	5	XLVIII	1	6	4	2
XXIV	1	6	0	6	XLIX	1	6	3	3
XXV	1	6	0	6	L	1	6	2	4
XXVI	1	6	1	5	LI	1	6	0	6
XXVII	1	1	0	1	LII	1	6	0	6
XXVIII	1	2	1	1	LIII	1	6	0	6
XXIX	1	5	0	5	LIV	1	6	5	1
XXX	1	6	0	6	LV	1	5	2	3
XXXI	1	4	0	4	LVI	1	6	1	5
XXXII	1	1	0	1	LVII	1	4	0	4
XXXIII	1	5	0	5	LVIII	3	6	0	6
XXXIV	1	6	0	6	LIX	1	6	0	6
XXXV	1	6	0	6	LV	2	6	0	6
XXXVI	1	6	0	6	LXI	2	6	0	6
XXXVII	1	6	0	6	LXII	2	4	1	3

How a seemingly pure female-laying female strain is obtained when only a few individuals are isolated from each generation while a parallel strain does not yield the same results is not yet clear. It may be due to some trick of selection in isolating the young females of each generation.

If a large number, 45 + per cent, of the daughter-females of one mother produce males it does not necessarily follow that a high or a low percentage of the daughter-females of the next generation will produce males. Nor does it seem to be true that if the daughter-females of one generation produce all female offspring that the daughter-females of the following generation will do so. Table XXII shows the history of both classes of daughter-females in five generations.

TABLE XXII

Record of all the female-laying females that were isolated in five consecutive generations, showing that there is no constant relationship between the percentage of male-laying females that are produced by a mother and the percentage of male-laying females that are produced by the daughter-female in the next generation.

Gen.	Sisters	Off-spring ♂ ♀	Moth- er ♀ ♀	Off-spring ♀ ♀	Gen.	Sisters	Off-spring ♂ ♀	Moth- er ♀ ♀	Off-spring ♀ ♀
I			I.		V	5	15		15
II		16	I.	36			1		34
							0		27
III	4	0		45			0		24
		2		42			5		14
		1		42					
		0		47					
			I.						
IV	5	12		20					
		4		41					
		0		36					
		7		40					
		22		26					
			I.						

VIII THE PRODUCTION OF FERTILIZED EGGS

The winter or fertilized egg is supposed to be the male parthenogenetic egg which has been fertilized. This produces a female. The egg has much more yolk material and a thicker shell than the male parthenogenetic egg. A female produces from twelve to twenty fertilized eggs, while a male-laying female produces from forty to fifty parthenogenetic eggs. In order to obtain fertilized eggs males must copulate with very young male-laying females.

In a few experiments, comprising several hundred females which had copulated with males when very young, Maupas found that the percentage of females producing fertilized eggs was the same as the percentage of male-laying females from several hundred females that never had copulated with males. He concluded that the fertilized egg is the male parthenogenetic egg which has been fertilized.

I have repeated his experiments on a smaller scale and the same results were obtained. Furthermore, the producers of fertilized eggs appeared among the early laid eggs of the mother-individuals.

Diagram 3 shows the occurrence of the layers of fertilized eggs among their sister-individuals from five mothers which produced 125 eggs.

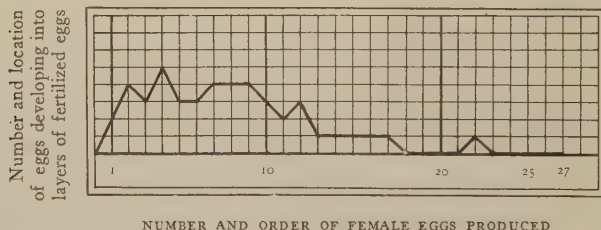


Diagram 3 Record of the egg production of five female-laying females showing in which part of the egg-laying period the layers of fertilized or "winter eggs" were produced. Each female laid 21 to 27 (average 25) eggs. Of the 125 daughter-females 36 + per cent laid fertilized eggs.

Many males, fifteen to twenty, were constantly kept in the dishes with each of the five mothers, so that as each daughter-female emerged from the egg there were many males present.

Only one male-laying female appeared among the 125 daughter-females. This experiment was conducted in the fourteenth generation of the strain in Tables I and XXI, soon after the isolation of the large numbers in generation XII which gave 21 + per cent of male-laying females.

Of the 125 daughter-females 36 + per cent produced fertilized eggs. This percentage is high because the mother-females produced, on an average, only twenty-five eggs each, but if they had produced forty eggs each the percentage would have fallen to

about twenty-three, provided that there had been no more mothers of fertilized eggs to have been produced. The Diagram 3 shows only one occurring between the eighteenth and twenty-seventh egg.

The number and order of occurrence of the mothers of fertilized eggs together with the number and occurrence of the layers of male eggs in parallel sets of daughter-females seem to indicate that the layer of male eggs and the layers of fertilized or winter eggs are identical at one stage of their life.

In another species of rotifer, *Asplancha*, Lauterborn has observed winter eggs and male embryos in the same individual. Among the *Daphnia*, Issakowitsch has found that the same female may produce winter eggs and male eggs.

Therefore it is not unreasonable to suppose that the immature male-laying female of *Hydatina senta* is capable of developing into a layer of fertilized eggs or a layer of male eggs, according to the impregnation or lack of impregnation by the male.

IX SUMMARY

1 Temperature has no influence in determining the sex of *Hydatina senta*.

2 About 22 per cent. of the females at any temperature from 14° to 29° C. are male-laying.

3 A male-laying female produces eggs faster than a female-laying female and at a temperature of 25° to 29° C. a male-laying female produces more eggs throughout her lifetime than a female-laying female.

4 The male-laying females occur in the early part of a family of daughter-females.

5 Starving the young females for the first few hours after they hatch does not cause them to produce a higher percentage of male eggs.

6 There are no strains that constantly produce a high or a low percentage of male-laying females.

7 The "pure female-laying female strain" can give rise to the normal percentage, $22 \pm$, of male-laying females.

8 The male-laying female may produce fertilized or winter eggs, provided that she has been impregnated by a male at the proper time.

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May 1, 1907

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THE EFFECT OF A CENTRIFUGAL FORCE UPON THE DEVELOPMENT AND SEX OF PARTHENO- GENETIC EGGS OF HYDATINA SENTA

BY

DAVID DAY WHITNEY

WITH ONE PLATE

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I. INTRODUCTION

The eggs of *Hydatina senta* are very favorable for experiments with the centrifugal machine. The adult females which contain eggs in stages up to and including the first maturation spindle may be centrifuged at the rate of twenty thousand revolutions in two to three minutes without any apparent injury. The animal is so transparent that the eggs can be seen immediately after centrifuging and their condition recorded. The materials in the egg are separated into three distinct zones, a pink zone, a middle clear zone and a gray zone. This stratified material only becomes partly redistributed in the egg before cleavage and sometimes scarcely any redistribution takes place. After the egg is laid the polar body is formed and the first cleavage appears in thirty-five to forty-five minutes. The eggs develop within forty-eight to seventy-two hours and produce in most cases normal embryos.

The following experiments and observations were made at the suggestion and under the supervision of Prof. T. H. Morgan.

II RELATION OF THE FIRST CLEAVAGE PLANE TO THE STRATIFICATION OF THE MATERIAL OF EGGS CENTRIFUGED WHEN THE GERMINAL VESICLE IS INTACT

If several hundred animals are centrifuged at the same time and are examined immediately many can be seen to contain eggs, the contents of which are sharply differentiated into three zones. As the animals are thrown down during the centrifuging process in any position the stratification of the eggs may lie in any relation to the median longitudinal axis of the female. The pink zone may be toward the head or foot of the animal, toward the side next the stomach or on the opposite side, or in any other intermediate position. The gray zone is always on the opposite side of the egg from the pink zone and the middle zone between the two. The germinal vesicle is found at the junction of the clear middle and pink zones and can be easily recognized owing to its large size (Fig. 1).

From the various lots of females, centrifuged twenty thousand revolutions, eight were selected containing the pink zone toward the head of the animal and the gray zone toward the foot. The germinal vesicle was plainly visible near the line between the clear middle and pink zones.

Each of these eggs was carefully watched while it remained in the oviduct of the female as well as after it was laid. In every egg the first cleavage appeared at that end of the egg which contained the pink pigment. The first division was unequal as it is in the normal egg. In some cases all the pink zone was cut off in the smaller cell while in other cases only a part of it was included in this cell. The gray zone was always included in the larger cell.

Six other females which had the pink zone of the egg toward the foot of the animal and the gray zone toward the head were isolated and the further history of each egg was carefully followed.

The first cleavage in all these eggs appeared in the end which contained the pink material.

It is hardly possible that in these fourteen eggs the pink material and the germinal vesicle were driven in each case to the animal pole. If this assumption is correct these two lots of eggs indicate that the position of the first cleavage is determined by the induced position of the germinal vesicle at either end of the egg. When, however, the germinal vesicle is carried to the side of the egg with the pink material, that is, when it comes to lie midway between the two ends of the egg, it moves later toward one of the ends of the egg since the first cleavage in such cases is at one end.

III RELATION OF THE FIRST CLEAVAGE PLANE TO THE STRATIFICATION OF THE MATERIAL OF EGGS CENTRIFUGED WHEN THE MATURATION SPINDLE IS PRESENT

Some of the centrifuged females were found containing eggs stratified into the three characteristic zones but showing the maturation spindle apparently at the side of the egg, where it normally forms, in the clear middle zone (Fig. 2).

In cases where the pink zone was in the end of the egg toward the head of the animal the first cleavage sometimes appeared in that end of the egg and cut off the pink zone in the small cell. At other times it appeared at the opposite end of the egg and included the gray zone in the small cell. In other cases where the gray zone was in the end of the egg toward the head of the animal the first cleavage sometimes appeared in that end but at other times it appeared in the opposite end of the egg. When the eggs were centrifuged so that the stratification was from side to side, instead of from end to end the first cleavage usually cut off a part of each of the three zones in each cell.

Apparently the first cleavage appeared at that end of the egg nearest to the maturation spindle irrespective of the stratified zones of the egg.

IV CONDITION OF THE YOUNG ANIMALS WHICH DEVELOPED:
FROM CENTRIFUGED EGGS

If the materials of the egg which are differentiated into zones by the centrifugal force are important formative substances their displacement and their different location in the first cleavage stage ought to cause abnormalities in the embryos and in the mature animals which develop from such eggs.

The following experiments which are only a few of those carried out will serve to indicate to what extent the embryos from centrifuged eggs were affected by the displacement of the materials.

Experiment I. January 11, 1908, at 8:30 p.m., many females were centrifuged about twenty thousand revolutions and then placed in a watch glass containing tap-water and allowed to lay their eggs. At 10 p.m., thirty-seven eggs were isolated.

January 13, at 9:30 a.m., there were in the dish thirty-two apparently normal young females, three winter eggs and two dead parthenogenetic eggs.

Experiment V (Control). January 21, at 10 p.m., two hundred and eighty eggs that had been laid by females which had not been centrifuged were isolated in a watch glass containing tap-water.

January 22, at 8 p.m., two hundred and forty normal young animals were removed from the dish.

January 23, at 11 a.m., six normal young removed and of the thirty-four unhatched eggs which remained thirty-one were winter eggs and three were dead parthenogenetic eggs.

Experiment XI. March 14, 7:45 p.m., several hundred females were taken from the culture jar and placed in a watch glass containing tap-water. No eggs had been laid by 8 p.m. These animals furnished material for the following experiments.

Lot A. At 8:35 p.m., twenty-five eggs which had been laid since 8 p.m., were isolated and centrifuged twenty thousand revolutions.

March 15, at 12:40 p.m., sixteen young normal females were taken out of the dish and nine unhatched eggs remained. At 9 p.m. the nine eggs were still unhatched. March 16, at 10 a.m., two of

the eggs had hatched. Of the seven remaining eggs one was a winter egg, three were dead parthenogenetic eggs and the other three contained living embryos which were apparently unable to break through the egg envelope.

Lot B. March 14, at 8:40 p.m., twenty eggs were isolated and allowed to remain undisturbed. At 9:10 p.m., these eggs were centrifuged in the same way and treated as Lot A. March 15, at 1:30 p. m., seventeen young normal females were in the dish and three unhatched eggs which contained living embryos. At 9 p.m., two of these eggs had produced normal females but the other was still unhatched.

Lot C. March 14, at 8:45 p.m., thirty eggs were isolated and allowed to remain undisturbed. At 9:45 p.m., these eggs, in some of which the first cleavage and in others the second and third cleavage had appeared, were centrifuged and treated as Lot A.

March 15, at 1:40 p.m., fourteen normal young females were removed from the dish and sixteen unhatched eggs remained. At 9 p.m., seven other normal young females were removed.

March 16, at 10 a.m., two other normal young females were removed. Seven eggs remained. One was a winter egg, two were dead parthenogenetic eggs and four contained living embryos.

Lot D (Control). March 14, at 10:30 p.m., thirty-three eggs were isolated.

March 15, at 12:30 p.m., thirteen normal young females and two normal young males were removed from the dish. At 9 p.m., seventeen other normal young females and one normal young male were removed.

In Experiment I the eggs were centrifuged when they contained the germinal vesicle or else the maturation spindle. In Experiment XI, Lot A, they were centrifuged in the stage when the polar body is forming or just after it was formed. In Lot B the eggs were centrifuged just before the first cleavage and in Lot C during and after the first cleavage.

In some of these experiments the young animals which developed from the centrifuged eggs seem to die sooner if not fed than do the animals developing from normal eggs. Also there

are more cases of the embryos unable to get out of the egg after they are fully formed. They can be seen writhing and twisting inside the egg and sometimes lived there as long as seven days.

These cases of abnormalities were due perhaps to the food material in the egg being displaced and therefore unable to nourish certain muscles which are well supplied with food material in the normal embryo. Consequently the young animal was weaker in certain parts of its body.

In a few cases the eggs began to develop but apparently soon ceased and never produced embryos which showed any ciliary movement. In normal embryos the ciliary movement around the head can be seen several hours before hatching. Whether the early death of such eggs is due to abnormal cleavage, misplaced egg substance, or misplaced chromosomes in division is not clear.

It is nevertheless apparent from these experiments that a very high percentage of normal young animals develop from eggs that have been centrifuged in the various stages of their early development.

V THE PROPORTION OF MALE AND FEMALE PRODUCING FEMALES IN THE FIRST, SECOND AND THIRD GENERATIONS AFTER CENTRIFUGING

If the dislocation of the egg substances has any influence on sex it should become evident by following the history of individual eggs in which the zones of stratification are differently arranged in their relation to the first cleavage plane.

The following data give the result of experiments carried out to examine the question.

Experiment XXXI. March 5, at 2:15 p.m., a female containing a large egg was centrifuged twenty thousand revolutions. At 3:10 p.m., the egg had been laid and was in the first cleavage stage. The pink zone was entirely included in the smaller cell (Fig. 3).

On March 6, at 11 a.m., a normal young female was swimming about in the dish. Food was then added. She produced eggs

and on March 8, at 10 a.m., four young daughter-females were present in the dish. This female produced twenty-nine eggs which developed into females. Two of these daughter-females matured and produced males and twenty-seven matured and produced females.

Experiment XXXIII. The conditions, size of egg, and the arrangement of egg material in the first cleavage were approximately the same as in Experiment XXXI. A normal young female developed from the egg, matured and produced males.

Experiment XXXVII. March 5, at 2:15 p.m., a female containing a large egg was centrifuged. At 4:30 p.m., the egg that had been laid was in the first cleavage stage. The small cell included portions of the pink and clear zones, Fig. 4.

On March 6, at 11 a.m., a normal young female was swimming about in the dish. Food was added. This female grew to maturity and produced twenty-five eggs, all of which developed into females. One of these daughter-females matured and produced males and twenty-four matured and produced females.

Experiment XLIV. The conditions, size of egg, and the arrangement of the egg material in the first cleavage were approximately the same as in Experiment XXXVII. A normal young female developed from the egg, matured and produced twenty-five eggs, all of which developed into female-laying females. In later generations males appeared.

Experiment XXXII. March 5, at 2:15 p.m., a female containing a large egg was centrifuged. At 3:30 p.m., an egg had been laid and was in the first cleavage stage. The small cell included portions of the three zones (Fig. 5).

On March 6, at 9 a.m., a normal young female was present in the dish. Food was added. This female matured and produced only three eggs. One of these eggs developed into a male-laying female and the other two developed into female-laying females. This small production of eggs was due to the scanty amount of food given to the female.

Experiment L. March 10, 1 p.m., a female containing a large egg was centrifuged. At 3 p.m., the egg had been laid and was in the first cleavage stage. The small cell contained about two-

thirds of the gray zone and a portion of the clear zone (Fig. 6). On March 11, at 11 a.m., a normal young female was swimming about in the dish. Food was added. This female matured and produced fifteen eggs which developed into females. One of these daughter-females produced males and the other fourteen produced females.

Experiment LV. The conditions, size of egg and the arrangement of the egg material in the first cleavage were approximately the same as in Experiment L. A normal young female developed from the egg, matured and produced fourteen eggs all of which developed into female-laying females. In later generations males appeared.

Several small male eggs were centrifuged in the same manner as the large eggs. In some of these the first cleavage plane appeared so as to cut off all the pink zone in the small cell and in others it cut off some of each of the three zones. In both cases apparently normal males were produced.

None of the females in the above experiments produced the normal number of eggs, which is forty to fifty, because of poor food conditions.

In former experiments it has been shown that the percentage of male-laying females in a family of daughter-females may vary from 0 to 50 per cent and also that the percentage of male-laying females in one generation is no indication what it may be in the next generation.

Experiments XXXI to LV. In these experiments the appearance of male-laying females from the various forms of centrifuged eggs does not seem to be markedly different from normal cases. In the experiments where the daughter-females of a family were all female-laying females males always appeared in the later generations, thus showing that no pure female-laying female strains were produced. Moreover, large (female) eggs never produced male animals nor did small (male) eggs ever produce female animals.

GENERAL DISCUSSION

Lyon has found in the eggs of the sea-urchin, *Arbacia*, that the first cleavage plane is always at right angles to the plane of stratification of the egg material.

Lillie has shown that the stratification, caused by centrifuged force, of the material in the egg of *Chætopterus* plays no part in determining the position of the polar lobe. When the germinal vesicle is still intact the egg has a well defined polarity, that is, one end is the animal and the other end the vegetative pole. If the germinal vesicle is driven to the vegetative pole the polar spindle which develops from it always migrates to the animal pole and there forms the polar bodies.

In later work on sea-urchin's eggs Morgan and Lyon show that, "while the cleavage conforms strictly to the induced stratification, the gastrulation does not conform to the symmetrical arrangement of the materials. The exceptional cases show that there is no necessary relation between stratification of the materials as such and the embryonic axes."

However, in the eggs of *Cumingia* Morgan has shown that the stratification of the egg material does not influence the position of the first cleavage plane. He says, "This difference is due to the shifting of the nucleus in the egg of the sea-urchin, while the spindle in *Cumingia* retains its original orientation."

It must be borne in mind that the above results were obtained from eggs centrifuged at different stages in their maturation. The eggs of *Chætopterus* were centrifuged when in the germinal vesicle and maturation spindle stages, while the eggs of *arbacia* were centrifuged when in the female pronucleus stage. The eggs of *Cumingia* were also centrifuged when in the maturation spindle stage and those of *Hydatina* in the germinal vesicle stage as well as in the maturation spindle stage.

When the eggs of these different animals are centrifuged in the maturation spindle stage the spindle is not usually moved from its original position and consequently the first cleavage take place precisely as it does in normal eggs.

When the eggs of *Chætopterus* and *Hydatina* are centrifuged

in the germinal vesicle stage the later histories of the germinal vesicles differ. In the egg of *Chaetopterus* the maturation spindle which develops from the germinal vesicle, according to Lillie, migrates to the animal pole if it does not happen to be located at that pole, while in *Hydatina* the maturation spindle never migrates from the end of the egg into which the germinal vesicle is driven by the centrifugal force. In the egg of *Arbacia* the female pronucleus may be so oriented by centrifugal force that the direction of the first cleavage plane is due rather to its location and follows in consequence the stratification of the materials in the egg.

None of the previous workers have reared the embryos from centrifuged eggs to maturity because the forms upon which they worked were not suitable for such experiments but *Hydatina* is an exceptionally favorable form for such work.

Eggs were centrifuged in various stages of maturation so that the zones of egg materials were differently arranged in their relation to the first cleavage plane, thus making it possible that in some cases the pink material of the egg would be included in the cells that make up the anterior end of the embryo while the gray material would be included in the cells of the foot region or vice-versa. In other cases the material would be more or less equally distributed in the anterior and posterior regions of the embryo.

In no case was the sex of the eggs changed and such eggs produced a very high percentage of normal young males and females. Furthermore the young females grew to adult animals and produced normal offspring of which the sex ratio was apparently normal.

It would, therefore, seem that the effect of centrifugal force upon the eggs of *Hydatina senta* is not sufficient to cause any noticeable change of structure or of sex in the animals that develop from them.

VI SUMMARY

I When the unsegmented eggs of *Hydatina senta* are centrifuged twenty thousand revolutions the materials in the eggs are stratified into a pink zone, a clear middle zone and a gray zone.

2 If eggs are centrifuged a short time before maturation when the nucleus is intact the nucleus is carried to the top of the clear zone against the bottom of the pink zone.

3 Very little redistribution of the egg materials takes place before the first cleavage. In consequence the segmented egg retains the distribution of materials impressed on it by the centrifugal force.

4 The first cleavage plane always appears at that end of the egg at which the pink zone and germinal vesicle are located. It forms across one end as in the normal egg separating a smaller and a larger cell.

5 The egg centrifuged after the polar spindle has formed shows that the spindle does not move from its original position. Its location determines the position of the first cleavage plane in so far as this appears at the end of the egg nearest to where the spindle lies.

6 Normal animals, both males and females, develop from centrifuged eggs and these have been reared to sexual maturity.

7 The sex of animals developing from large (female) or small (male) eggs is not affected by the centrifugal force nor is the sex ratio in the descendants of females developing from centrifuged eggs altered.

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April 25, 1908

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DESCRIPTION OF PLATE

Fig. 1 Section of a centrifuged egg, showing the three zones and the germinal vesicle located near the boundary of the pink and the clear zones. Gilson mercurio-nitric fixing fluid and Heidenhain's iron hæmatoxylin stain.

Fig. 2 Section of a centrifuged egg showing the three zones and the maturation spindle in the clear zones. Yolk granules are lodged against the achromatic figure. Bouin's fixing fluid and Heidenhain's iron hæmatoxylin stain.

Figs. 3 to 6 Free hand semi-diagrammatic drawings of living eggs in the first cleavage stage which were centrifuged when in the oviducts of the females. The fine stippling shows the position of the pink zone, the coarser stippling shows the position of the gray zone, and the clear space indicates the clear zone.

Fig. 3 Experiment XXXI—The pink zone included in the small cell and the gray and clear zones are in the larger cell.

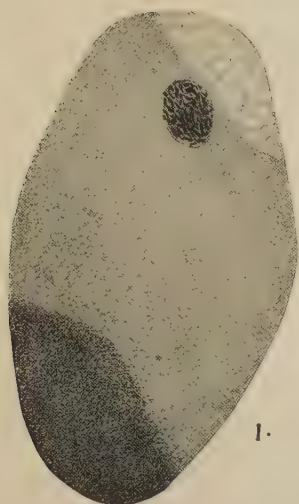
Fig. 4 Experiment XXXVII—About one-half of the pink zone and a portion of the clear zone included in the small cell and the other part of the pink zone, all the gray zone, and a part of the clear zone are included in the larger cell.

Fig. 5 Experiment XXXII—Portions of each of the three zones in each of the two cells.

Fig. 6 Experiment L—About two-thirds of the gray zone included in the small cell while the other third of the gray zone together with all of the pink zone and nearly all of the clear zone are included in the larger cell.

EFFECT OF A CENTRIFUGAL FORCE UPON DEVELOPMENT AND SEX

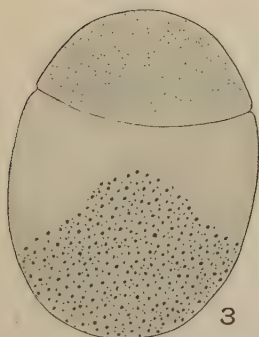
DAVID DAY WHITNEY



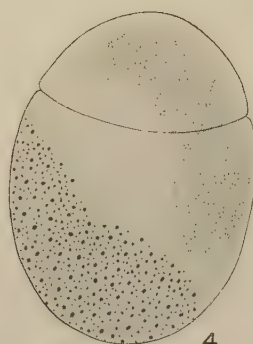
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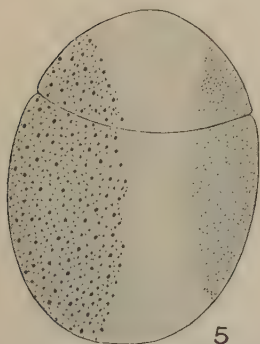
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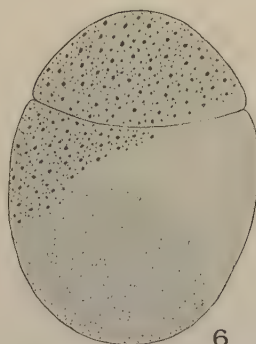
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6

OBSERVATIONS ON THE MATURATION STAGES OF THE PARTHENOGENETIC AND SEXUAL EGGS OF HYDATINA SENTA

BY

DAVID DAY WHITNEY

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I INTRODUCTION

Despite the experiments that have been carried out by several workers to discover how sex is determined in parthenogenetic eggs the attempts to show that such external factors as temperature or food influence the result do not appear to have been successful. Attention has turned more recently to the possibility that there are internal factors in the eggs that are all-important in producing males or females.

As early as 1845 Dzierzon brought forward very strong evidence to show that the eggs of the honey-bee, *Apis mellifica*, always develop into males if unfertilized, but if fertilized they develop into females (queens or workers). In other words, internal rather than external agents bring about the result. This theory has been often attacked and strongly defended, and now seems to be generally accepted.

In the aphids Balbiani and Stevens find that the same female may produce both male parthenogenetic and fertilized or winter eggs. Lauterborn finds the same phenomenon in the Rotifer, *Asplanchna*, and Issakowitsch in a Daphnid. Whether the eggs that are fertilized are originally male eggs or develop from a dif-

ferent part of the ovary is not certain, but the evidence seems to indicate that they are male eggs.

In the two kinds of parthenogenetic eggs of some of the aphids Stevens finds that there is no reduction in the number of the chromosomes during the formation of the polar body. Lenssen, in a study of the parthenogenetic eggs of the Rotifer, *Hydatina senta*, finds that there is a reduction in the number of the chromosomes during the formation of the polar body in the male egg but no reduction in the female egg.

Weismann found that both kinds of parthenogenetic eggs of *Polyphemus* (Daphnid), certain Ostracods and Rotifers produced only one polar body while the fertilized eggs produced two polar bodies. Blochmann and Stevens found the same relation to hold for certain aphids.

In *Lisparis dispar*, a parthenogenetic Lepidopteran, Platner found that two polar nuclei were formed. In the bee *apis*, Blochmann, Paulcke, Petrunckewitsch and others find that the parthenogenetic eggs which develop into male animals give off two polar bodies. In the Rotifer, *Asplanchna*, Mrazek, Erlanger and Lauterborn found that the female parthenogenetic egg gave off one polar body and that the male parthenogenetic as well as the fertilized egg gave off two polar bodies. In the parthenogenetic eggs of *Hydatina senta* Lenssen thought that the male egg gave off one polar body and the female egg gave off none!

At the suggestion and under the supervision of Prof. T. H. Morgan the following work upon the eggs of *Hydatina senta* was done with the view of obtaining more light upon the maturation stages and their relation to the determination of sex.

I am also indebted to Prof. E. B. Wilson for many valuable suggestions and criticisms.

II MATERIAL AND METHODS

The Rotifers were collected and reared in cultures as described in a former paper.

The first maturation spindle is formed before the egg is laid and in order to study the early maturation stages animals con-

taining eggs were killed and fixed in masses of thousands and sectioned in toto. Many eggs were found in the desired stages, but as the eggs are filled with yolk granules of various sizes it was exceedingly difficult to find many sections in which the yolk granules were not mingled with the chromosomes.

Hot sublimate acetic, Bouin's fluid, strong Flemming, Gilson, Carnoy, and alcohol acetic, were used as killing and fixing fluids. Some good preparations were obtained by each method, but alcohol acetic gave the best results in obtaining equatorial plates; for it coagulated the cytoplasm of the egg in such a way as to embed the yolk granules in its meshes, thus leaving the spindle and its chromosomes free from yolk granules. Thousands of animals were sectioned and about three hundred good slides were made. Sections were cut 5μ in thickness in 51° to 52° C. paraffine.

Many parthenogenetic females were also isolated separately and the sex of their offspring determined, for those eggs first laid, before the females were killed and sectioned. The general nature of the maturation stages of such eggs was determined before a more detailed study was made of the eggs in the mixed slides.

After the eggs are laid the envelope around them is so thin and at the same time so exceedingly impervious to fixing fluids that the eggs usually collapse in the process of fixation. Sometimes a few do not collapse in alcohol acetic but, however careful one may be, by the time the eggs are embedded they have shrunk. In such eggs the yolk granules are so crowded in among the chromosomes and stain so darkly that no satisfactory results can be obtained.

In order to free the spindle from these granules the eggs were first centrifuged. In sections of such eggs the maturation spindle remained in the clear middle zone of the egg and was often entirely free from yolk granules. As only a few sections of these eggs were made no good stages were found in which the chromosomes could be counted but the method gives promise of results that can not be obtained in other ways.

Heidenhain's iron hæmatoxylin was used chiefly and gave the best results although many other stains were tried.

In order to see the polar bodies the eggs, some time after they

were laid, were put into Schneider's aceto-carmine for about thirty seconds and then into a water-glycerine solution (1 drop in 5 cc. of water). The blastomeres become separated and the polar bodies can be readily seen.

III FEMALE EGG

The female egg is easily distinguished from the male egg by its larger size and is never mistaken for the winter egg which may be of equal size, but has a much thicker envelope around it, besides containing the conspicuous sperm nucleus.

In the female parthenogenetic egg the number of chromosomes was never definitely determined but many spindles in metaphase were seen in side view, containing numerous chromosomes (20 to



Fig. 1 Female parthenogenetic egg. *A*, equatorial plate of the polar spindle, showing twenty-three to twenty-five chromosomes; *B*, prophase of polar spindle, showing twenty-two chromosomes.

30). In one polar view of a metaphase twenty-five chromosomes were seen, Fig. 1, *A*. In a prophase twenty-two dumb-bell shaped chromosomes were seen in one section (Fig. 1, *B*) and in the adjoining section there were four other dumb-bell shaped chromosomes together with one that was not constricted. No anaphase or telophase stages were found although hundreds of eggs were examined. Lenssen found the chromosomes somewhat scattered about on the equator of the maturation spindle and concluded they were in an early anaphase but since he considered the unreduced number to be ten or twelve chromosomes the twenty or more chromosomes that he saw were probably in an early meta-

NOTE—The drawings of the chromosomes were made as carefully as possible with a camera under a 1.5 mm. Zeiss apochromatic and compensation ocular 6. They were then enlarged with a drawing camera about three times, corrected by comparison with the objects, and reduced by one-third in reproduction.

phase instead of in an early anaphase. He never saw a telophase stage and decided without any evidence that the chromosomes never separated beyond the early anaphase stage and that later all the chromosomes form the segmentation nucleus.

This is probably not the case because one polar body can always be seen near the periphery of the egg after the first cleavage, in total mounts prepared by the method already described. Sometimes a constriction can be seen across the middle of the polar body giving it the appearance of being divided into two parts. In the two-cell stage of the egg after the two blastomeres separate the polar body is always found in the space between the two cells (Fig. 2, *A-B*). In the four-cell stage it is seen at the point of juncture of the four cells (Fig. 2, *C*).

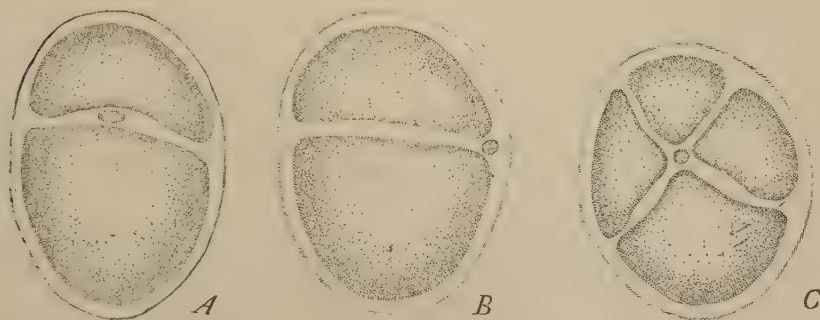


Fig. 2 Female parthenogenetic egg. *A, B*, eggs in the two-cell stage, showing one polar body; *C*, egg in the four-cell stage, showing one polar body at the intersection of the two cleavage planes.

IV MALE EGG

The male egg is much smaller than the other two kinds of eggs and has a thin envelope around it similar to that of the female parthenogenetic egg. The maturation spindle was seen several times when the chromosomes were in metaphase, anaphase and telophase stages. In two cases of telophase ten and fourteen chromosomes respectively were counted on one end of the spindle (Fig. 3, *C-D*). Polar views of the metaphase stage showed clearly eleven to thirteen chromosomes (Fig. 3, *A-B*). They were always less in number and larger in size than the chromosomes in the metaphase stage of the female parthenogenetic eggs.

Three polar bodies are to be found near the periphery of the egg close to the line of meeting of the blastomeres. One was usually larger than the other two and often at a little distance away from them (Fig. 4, *A-B*), although in one instance the three polar bodies were close together and seemed to be of the same size (Fig. 4, *C*).



Fig. 3 Male parthenogenetic egg. *A, B*, equatorial plates of the polar spindle, showing twelve to thirteen chromosomes; *C, D*, polar spindle in telophase, showing ten to fourteen chromosomes.

Lauterborn states that in the male parthenogenetic egg of *Asplanchna* the first of the two polar bodies which was extended usually divided.

Lenssen concluded that only one polar body was formed because he saw the maturation spindle in the telophase stage. He did not follow the history of the chromosomes in the later stages and consequently never saw any polar bodies.



Fig. 4 Male parthenogenetic egg. *A, B*, eggs in the four-cell stage, showing three polar bodies, two of which are smaller than the other; *C*, egg in the two-cell stage, showing three polar bodies of nearly the same size.

V WINTER EGG

The fertilized or winter egg has a very thick envelope. An oval shaped small body which is probably the sperm nucleus is always found near the egg nucleus.

The chromosomes were seen in sections on the maturation spindle (side view) in all stages but in only two anaphase stages (Fig. 5,

C-D), could they be counted because of being too closely crowded together. The polar view of the metaphase in the alcohol acetic fixation gave the best results. Fourteen chromosomes were seen in several sections of different eggs (Fig. 5, A-B). The chromosomes were of about the same size as those in the metaphase of the male parthenogenetic egg and were much larger in size and less in number than those in the metaphase of the female parthenogenetic egg.



Fig. 5 Winter or fertilized egg. A, B, equatorial plates of the polar spindle, showing fourteen chromosomes; C, D, anaphases of the polar spindle, showing twelve to fourteen chromosomes on each end of the spindle.

On account of the thick and opaque egg envelope, the polar bodies were never seen.

VI GENERAL DISCUSSION

Although Lenssen was mistaken in regard to the number of the chromosomes nevertheless he was firmly convinced that the number in the maturation stages of the male parthenogenetic and the fertilized egg were the same and that the number in the female parthenogenetic egg was greater. By comparing my Figs. 1, 3 and 5, it will be seen that this conclusion is confirmed. The greatest number of chromosomes seen in an equatorial plate of the male egg was possibly thirteen and the number seen in an equatorial plate of a winter egg was fourteen. The chromosomes of both eggs in the same stages were of the same size.

In the female parthenogenetic egg the greatest number of chromosomes seen was twenty-five (Fig. 1). The chromosomes were

very much smaller than in the other two kinds of eggs and usually were so crowded together that it was impossible to count them except in a very few cases.

These observations show that there is probably a reduction in the number of chromosomes in the male parthenogenetic and winter egg but no reduction in the female parthenogenetic egg. The former case would be similar to what occurs in the honey-bee. In the aphids Stevens found that there is no reduction in the number of chromosomes in either of the male or female parthenogenetic eggs but only in the fertilized egg.

It appears that in different animals parthenogenetic eggs vary in the number of polar bodies that they give off. The male egg of *Asplanchna*, *Hydatina* and *Apis* gives off two polar bodies while the male egg of aphids gives off only one.

If it is true that the male egg when fertilized becomes the winter egg which develops into a female it seems evident that the reduction in the number of chromosomes and the formation of the second polar body is not in itself the factor that determines the ultimate sex of the egg.

The sperm would seem to introduce a factor that determines the sex of the embryo. This idea is strongly suggested by the evidence that Meves has brought forward in the case of the honey-bee in which he finds that only one kind of functional sperm is produced. Morgan also finds a similar phenomenon for certain Phylloxerans. If the same process occurs in the sperm of *Hydatina* the cause of the change in sex of the male egg may be at least surmised.

VII SUMMARY

- 1 In the female parthenogenetic egg of *Hydatina senta* there is no reduction in the number of chromosomes during maturation. One polar body is extruded.

- 2 In the male parthenogenetic egg there is a reduction in the number of chromosomes during maturation. Two polar bodies are formed, one of which subsequently divides.

- 3 In the winter egg, that becomes fertilized, there is a reduction in the number of chromosomes during maturation, and since

a similar process of reduction takes place in the parthenogenetic egg that becomes a male it would seem to follow that the sex of the embryo from this egg is changed by the spermatozoön.

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April 25, 1908

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